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Immunology

Ia-transfected L-cell fibroblasts present a lysozyme peptide but not the native protein to lysozyme-specific T cells

(antigen presentation/antigen processing/T-cell activation)

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ABSTRACT **We studied the antigen-presenting capacity of mouse L fibroblasts transfected with genes encoding Ia polypeptides of the major histocompatibility complex (MHC). These cells function as efficient antigen-presenting cells (APC) in stimulating peptide antigen-specific MHC-restricted proliferation of long-term T-cell lines, thus establishing the capacity of Ia-expressing L-cell transfectants to present antigens to apparently normal T cells. However, in contrast to splenic APC, L-cell transfectants fail to present native hen egg-white lysozyme to the same T cells. Since this result is similar to that obtained with physiologic APC pretreated to prevent antigen degradation, it suggests that L-cell transfectants, without such pretreatments, may be compromised in their ability to process native lysozyme. However, since such transfectant cells have been shown to present other complex polypeptides such as keyhole limpet hemocyanin, a random copolymer of glutamic acid, alanine, and tyrosine, and influenza virus neuraminidase, this observation suggests that protein antigens differ in the stringency of processing requirements.**

The I-A and I-E molecules, together known as the Ia molecules, are encoded within the major histocompatibility complex (MHC). These molecules serve key functions in regulating the activation of T helper lymphocytes. Phenomena such as restriction of antigen recognition, alloreactivity, and many immune response gene defects are known to be profoundly influenced by the extremely polymorphic Ia molecules (for reviews, see refs. 1 and 2). T cells can be stimulated to proliferate or secrete lymphokines, such as interleukin 2 (IL-2), only by antigen-presenting cells (APC) expressing the appropriate Ia molecule. In most instances, T-cell stimulation requires both antigen and a particular Ia molecule. Structural changes in the Ia molecules, such as those that occur naturally as polymorphic differences among inbred *H-2* recombinant mouse strains, do not lead to T-cell stimulation, even when the same antigen is employed. This strict specificity for a particular *Ia* allele is known as MHC restriction of antigen recognition. The structural features of the α and β subunits of the Ia molecules, relevant to MHC-restricted activation, are not well defined.

Recent advances in cloning the genes of the α and β subunits of the Ia molecule and their expression in cells that normally do not express *Ia* gene products have allowed a direct approach to the study of the structure–function relationships of the Ia molecule (3–8). It has been shown that mouse fibroblasts transfected with the genes of the murine Ia or human DR polypeptides can express the molecules on the cell surface. Moreover, these Ia-expressing transfectants are capable of stimulating several antigen- and allo-specific T cells in an MHC-restricted manner (5–8).

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In this report, we extend these studies to long-term antigen-specific T-cell lines to demonstrate that both I-A^k- and I-E^k-expressing L-cell transfectants present a lysozyme peptide in an MHC-restricted manner. For I-E^k-restricted T cells, the E_β subunit was found to determine the MHC restriction specificity. However, the same transfectant cells were incapable of presenting native lysozyme to the same T cells. This result is similar to the results obtained with APC pretreated with either lysosomotropic drugs or aldehydes; such cells were also found to present peptides derived from protein antigens but not the native proteins themselves (9–11). Thus it would appear that Ia-expressing mouse fibroblasts, though capable of providing all of the specificity requirements for normal T-cell activation, are compromised in their ability to process native lysozyme.

MATERIALS AND METHODS

Mice. CBA/J and C3H/HeJ mice were obtained from The Jackson Laboratory and bred in our colony. Animals of either sex were used at 3–12 months of age.

Antigens. Purified hen egg-white lysozyme (HEL) was a kind gift of A. Miller and E. Sercarz (University of California, Los Angeles). The T11(H) peptide (amino acids 74–96 of HEL) was prepared by solid-phase synthesis as described elsewhere (12).

T cells. The T-cell lines and clones used in this study are listed in Table 1. These long-term T-cell lines and T-cell hybrids were generated according to described methods (12–14).

Transfected Fibroblast Cell Lines. The parental Ltk^- cell line and five different transfected cell lines were used in this study (Table 1). The transfectants were generated by cotransfer of the genes of Ia subunits and the thymidine kinase (tk) gene as a selectable marker in the mouse Ltk^- fibroblast cell line. The transfection procedures and selection of clones CA14.11.14, CA14.11.29, and CA14.11.35 have been described extensively (3, 6). The I-E-expressing cell lines were constructed in a similar way using the E^k_α and E^d_β genes (CA36.2.1) or the E^k_α and $E^{d/k}_\beta$ genes (CA36.1.3). The $E^{d/k}_\beta$ gene was derived from a construct consisting of the leader exon from the E^d_β gene and the remainder of the molecule from the E^k_β gene (unpublished data). As the leader peptide is excised prior to cell surface expression (leaving three apparently silent amino acid substitutions in the E^k_β NH_2-terminal region), and the E^k_α and E^d_α subunits are almost identical (15, 16), the expressed $E^{d/k}_\beta$ and $E^k_\alpha E^d_\beta$ molecules are referred to here as E^k_β and I-E^d, respectively.

The cell surface expression of the expected determinants of the I-E molecules on these two cell lines was analyzed by

Abbreviations: APC, antigen-presenting cells; HEL, hen egg-white lysozyme; IL-1 and IL-2, interleukin 1 and interleukin 2; MHC, major histocompatibility complex; T11(H), amino acids 74–96 of HEL.

Table 1. Cell lines used in this study

Designation	Cell type	Mouse strain of origin	Antigen specificity	MHC restriction	Ia expression
KOIT	Long-term bulk T line	CBA	T11(H)	I-A^k	
AOIC	Long-term bulk T line	B10.A	T11(H)	I-E^k	
KOIT.2.10	Long-term T clone	CBA	T11(H)	I-A^k	
AOIT.H.2	T hybridoma	B10.A T cells fused with AKR thymoma (BW5147)	T11(H)	I-A^k	
Ltk^-	Fibroblast	C3H			None
CA14.11.14	Transfectant fibroblast	C3H			I-A^k
CA14.11.29	Transfectant fibroblast	C3H			I-A^k
CA14.11.35	Transfectant fibroblast	C3H			I-A^k not detectable
CA36.1.3	Transfectant fibroblast	C3H			I-E^k
CA36.2.1	Transfectant fibroblast	C3H			I-E^d

using the available monoclonal antibodies (17). Table 2 shows that both CA36.1.3 and CA36.2.1 cells expressed the widely cross-reactive determinants detected by antibodies 10.B and 39.I. However, only CA36.1.3 cells reacted with antibody 9.A, which is specific for the I-E^k molecule. This demonstrates that the two cell types express different serological determinants corresponding to the transferred genes. The control antibodies 84.17.2 and 10.2.16, respectively directed towards the endogenously expressed K^k molecule and the unexpressed I-A^k molecule, showed the expected reactions.

Cell Cultures. The fibroblast cell lines were maintained in α-MEM (GIBCO) supplemented with 2 mM glutamine, 1 mM pyruvate, 50 μM 2-mercaptoethanol, penicillin at 100 units/ml, streptomycin at 100 μg/ml, and 10% fetal bovine serum (Irvine Scientific, lot 404248). All other cell lines and cultures were maintained in similarly supplemented RPMI 1640 medium (GIBCO). The cultures were incubated at 37°C in an atmosphere of 5% CO_2/air.

T-Cell Stimulation Assays. Specific stimulation of long-term T-cell lines was assessed in a proliferation assay. To reduce background proliferation, APC (freshly prepared spleen cells or transfected L fibroblasts) were treated with mitomycin C (Sigma) at 50 μg/ml at 37°C for 30 min and were washed four times with Hanks' balanced salt solution (BSS) before use. T cells (1×10^4), purified by passage through Ficoll-Paque (Pharmacia), were cultured with mitomycin C-treated spleen cells (5×10^5) or L fibroblasts (1×10^5) in the presence of antigen or medium alone. Incorporation of 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine added per culture (30 Ci/mmol; ICN) was assessed during the last 48 hr of a 3-day culture. In some experiments, monoclonal anti-Ia antibody-containing culture supernatants of 10.2.16 or 14.4.4 cells were included in the cultures.

Stimulation of T-cell hybrid AOIT.H.2 was assessed by estimating the IL-2 content in a 24-hr supernatant of 1×10^5 T cells cultured with APC and antigens in a total volume of 0.2 ml. Fifty microliters of the supernatant was added to 5×10^3 cells, in 50 μl of medium, of the IL-2-dependent cell line HT-2 (kindly provided by J. Kappler and P. Marrack, Denver, CO) (14). Growth of the cells was estimated by [^{3}H]thymidine incorporation during the last 4–8 hr of a 24-hr culture.

Table 2. Serological analysis of the Ia molecules present on the surface of the L-cell transfectant lines CA36.1.3 and CA36.2.1

Monoclonal antibody*	Specificity	Known cross-reactions	^{125}I-protein A bound,† cpm $\times 10^{-3}$ CA36.1.3 (E^k_α, E^k_β)	CA36.2.1 (E^k_α, E^d_β)
10.B	I-E^k, Ia.7	d, v, r, p, u, j	270	279
39.I	I-E^k	d, v, r, p, u, j, b, q	216	278
9.A	I-E^k	None	197	1.3
84.17.2	K^k		417	393
10.2.16	I-A^k, Ia.17		0.6	0.5

*The monoclonal antibodies used here were kindly provided by Michel Pierres. The specificities of these antibodies have been described (17).

†Transfectant cells (5×10^5) were incubated with 50 μl of the appropriate monoclonal antibody (10 μg/ml). After 1 hr at 4°C the cells were washed twice, resuspended with 10^5 cpm of ^{125}I-labeled staphylococcal protein A, and incubated for an additional 45 min at 4°C. Finally, after three washes, cell-associated radioactivity was measured in a γ counter.

RESULTS AND DISCUSSION

I-A^k-Expressing Mouse Fibroblasts Can Serve as APC for Long-Term Antigen-Specific T-Cell Lines. Mouse L-cell fibroblasts do not normally carry out the functions associated with APC for T-cell stimulation. However, when these L cells are transfected with the genes for the Ia molecules, they can express the Ia molecule on the cell surface and serve as APC for MHC-restricted antigen-specific T-cell hybrids (5–8). T-cell hybrids are known to be less stringent, compared to normal T cells, in their accessory requirements such as interleukin 1 (IL-1) for stimulation (18). It is possible that while the expression of Ia molecule on L cells could be a sufficient condition for stimulation of T-cell hybrids, the same L cells may not be capable of presenting antigens to normal T cells, because of an inherent deficiency in providing the accessory requirements. We assessed the APC function of the I-A^k-expressing L-cell transfectant CA14.11.14 to present the T11(H) peptide (amino acids 74–96 of HEL) to long-term T-cell lines specific for this peptide and restricted by the I-A^k molecule (Table 3). For both the bulk line KOIT and the cloned line KOIT.2.10, the I-A^k-expressing CA14.11.14 cells were found to be efficient APC. The untransfected Ia^- Ltk^- cells could not serve as APC, indicating the necessity of Ia expression. Considering that the APC constitute a very small (<1%) fraction of the heterogeneous spleen cell population, it should be noted that on a per cell basis CA14.11.14 cells were lower in their stimulatory capacity. The stimulation was, however, always significant and reproducible. Recently, it was shown that murine Ia- or human DR-expressing transfectant L cells can present peptides of pigeon cytochrome *c* or viral neuraminidase to long-term T-cell lines (7, 8). Taken together, these results show that independently generated Ia-expressing L cells can serve as APC for presentation of peptides to apparently normal T cells and are thus capable of providing all the accessory requirements usually associated with physiologic APC. The interesting issue raised by these studies is the role

Table 3. Mouse L-cell fibroblasts transfected with the genes of the Ia molecule can serve as efficient APC for long-term antigen-specific T-cell lines

	[^{3}H]Thymidine incorporation, cpm × 10^{-3} per culture					
	Ltk$^-$ cells		CA14.11.14		CBA spleen	
T cells	Medium	T11(H)	Medium	T11(H)	Medium	T11(H)
KOIT	0.3 ± 0.1	0.2 ± 0.1	10.0 ± 0.3	*68.2 ± 0.6*	0.5 ± 0.2	*256.3 ± 17.8*
KOIT.2.10	0.2 ± 0.1	0.2 ± 0.1	10.3 ± 0.6	*57.2 ± 1.2*	3.5 ± 4.2	*247.6 ± 3.1*

T cells (1×10^4) passed through Ficoll-Paque were cultured with mitomycin C-treated syngeneic CBA spleen cells (5×10^5), I-A^k-transfected L cells (CA14.11.14) (1×10^5), or untransfected L cells (Ltk$^-$) (1×10^5), in the absence (medium alone) or presence of 7 μM T11(H) peptide. Incorporation of 1 μCi per culture of [^{3}H]thymidine was assessed during the last 18 hr of the 3-day culture. Results are mean ± SEM.

of IL-1 and other accessory molecules (such as L3T4, etc.) considered essential for normal T-cell activation (reviewed in ref. 19). It is presently unclear whether mouse fibroblasts are capable of providing such functions or whether these requirements are bypassed.

Antigen Presentation by Ia-Expressing Transfectants Is MHC Restricted. The specificity of Ia-mediated antigen presentation function of L-cell transfectants was assessed by using T cells restricted by either the I-A^k or the I-E^k molecule. Table 4 shows that the splenic APC from the CBA mouse (expressing both the I-A^k and I-E^k molecules) can present the T11(H) peptide to both the I-A^k-restricted clone KOIT.2.10 and the I-E^k-restricted AOIC bulk T-cell line. The ability of the transfectant fibroblasts to present the same antigen to the T cells was dependent upon the expression of the appropriate Ia molecule. Thus, I-A^k-restricted KOIT.2.10 cells were stimulated by I-A^k-expressing CA14.11.14 and 14.11.29 cells but not by CA36.1.3 cells, which express the I-E^k molecule. Conversely, I-E^k-restricted AOIC T cells could be stimulated only by CA36.1.3 cells and not by any of the I-A^k-expressing transfectants. The CA14.11.14 cells express higher levels of cell surface I-A^k molecules compared to CA14.11.29 cells and show a higher stimulatory capacity (6). Transfectant CA14.11.35 (thymidine kinase-positive) cells do not express detectable levels of Ia and were quite incapable of serving as APC, suggesting a correlation between the quantitative expression of cell surface Ia molecules and the stimulatory capacity of the transfectants. Thus, in agreement with the earlier studies, the transfection procedure does not by itself convert the fibroblasts into APC, but requires the expression of cell surface Ia molecules.

Further evidence for the direct involvement of the Ia molecule in the APC function was obtained by using monoclonal anti-Ia antibodies to inhibit this function. Proliferation of I-A^k- and I-E^k-restricted KOIT.2.10 and AOIC T cells, induced by the T11(H) peptide, in the presence of the appropriate Ia-expressing L cells, could be reciprocally inhibited by antibodies directed towards either the I-A^k or the I-E^k molecules (Table 5). This demonstrates the direct involvement of the Ia molecules in APC function and, taken together with the correlation between stimulatory capacity and quantitative surface expression (20), supports the view that the antigenic peptide and the Ia molecule together constitute all of the specificity requirements necessary for T-cell receptor recognition (1, 14). This has recently been elegantly confirmed by the success in antigen-specific stimulation of T-cell hybrids by using purified Ia molecules incorporated in planar membranes (21).

Restriction Specificity of the I-E Molecule Is Determined by the β Subunit. One of the advantages in using the transfected mouse fibroblasts instead of other tumor cell lines as APC (22, 23) lies in the ability to manipulate predetermined structural features of the Ia molecule by altering the genes used for transfection. Such transfectants, expressing specifically altered Ia molecules, would be invaluable in mapping the structural features of the Ia molecule relevant to the MHC-restricted activation process. Moreover, functional studies have suggested that the Ia molecules exhibit multiple restriction sites (24, 25). However, the location of these sites on the Ia molecule is not known. As a beginning in the analysis of structural correlates of Ia function, we transfected mouse L-cell fibroblasts with the genes for either the E^k_α and E^k_β (cell line CA36.1.3) or the E^k_α and E^d_β (CA36.2.1) molecules. Since the E_α molecules of the *k* and *d* haplotypes are almost identical (16), it was expected and shown by serological analysis with monoclonal antibodies that CA36.1.3 and CA36.2.1 cells express determinants expected of the I-E^k and I-E^d molecules, respectively (Table 2). Table 6 shows that the antigen-induced proliferative response of I-E^k-restricted AOIC T cells could be obtained only with $E^k_\alpha E^k_\beta$-expressing CA36.1.3 and not with $E^k_\alpha E^d_\beta$-expressing CA36.2.1 cells. The latter CA36.2.1 cells have been shown to stimulate antigen-specific I-E^d-restricted T-cell hybrids (unpublished data). This confirms the previous genetic and structural analyses and suggests that the restriction specificity of the I-E molecule correlates with the more polymorphic β subunit (26).

Table 4. L-cell antigen presentation function requires the expression of the appropriate gene product

			[^{3}H]Thymidine incorporation, cpm × 10^{-3} per culture			
			KOIT.2.10		AOIC	
APC	Ia expression		Medium	T11(H)	Medium	T11(H)
CBA spleen	I-A^k	I-E^k	1.9 ± 1.9	*108.4 ± 5.6*	1.9 ± 3.6	*207.3 ± 10.1*
CA14.11.14	I-A^k	—	2.6 ± 0.3	*76.6 ± 2.3*	2.0 ± 0.3	8.7 ± 0.8
CA14.11.29	I-A^k	—	2.8 ± 0.1	*44.5 ± 1.7*	2.7 ± 0.2	5.3 ± 0.2
CA36.1.3	—	I-E^k	12.0 ± 0.2	7.1 ± 0.2	12.3 ± 3.3	*110.2 ± 4.0*
CA14.11.35	—	—	4.9 ± 0.1	4.7 ± 0.1	4.7 ± 0.3	5.5 ± 0.4

T11(H)-specific I-A^k-restricted KOIT.2.10 and I-E^k-restricted AOIC T cells were assayed for antigen-induced proliferative response, using different Ia-transfected L cells and CBA spleen cells as APC. Culture conditions were identical to those in the legend to Table 3.

Table 5. Ia L-cell antigen presentation function involves the expressed Ia molecule

Monoclonal antibody	Specificity	[^{3}H]Thymidine incorporation, Δcpm $\times 10^{-3}$ per culture	
		KOIT.2.10	AOIC
—	—	*114.9 ± 2.3*	*160.1 ± 2.4*
10.2.16	I-A^k	4.0 ± 1.4	*135.3 ± 3.9*
14.4.4	I-E^k	*128.1 ± 4.5*	7.3 ± 0.2

I-A^k-restricted KOIT.2.10 and I-E^k-restricted AOIC cells were cultured with, respectively, I-A^k- and I-E^k-expressing CA14.11.14 and CA36.1.3 L cells in the presence of 7 μM T11(H). Culture conditions were identical to those in the legend to Table 3. Background cpm with medium alone were 3.6×10^3 for KOIT.2.10 and 7.9×10^3 for AOIC; these have been subtracted. Monoclonal antibodies 10.2.16 and 14.4.4 were included in the cultures at a dilution of 1:100.

The generality of this conclusion for other antigens and a more precise definition of the relevant regions of the E_β subunit affecting reactivity must await further studies.

Ia-Transfected Fibroblasts Fail to Present Native Lysozyme to T Cells. While it is clear that the Ia molecule serves a specific role in the activation of T cells, the antigen presentation phenomenon involves events that must occur prior to the recognition event (reviewed in ref. 27). Such events, referred to as antigen processing, have been distinguished from the recognition event by the use of either lysosomotropic drugs or aldehyde-fixed APC. Under these conditions, it has been found that APC fail to present native protein antigens, whereas the presentation of smaller peptides, and in some cases denatured proteins, to T cells is not affected (9–11). The molecular mechanisms through which the APC degrade and eventually present antigen fragments in the context of the Ia molecule on the cell surface apparently involve lysosomal pathways that are poorly understood (27).

In the other studies, T-cell responses to large complex polypeptide antigens such as keyhole limpet hemocyanin, random polymer of glutamic acid, alanine, and tyrosine (GAT), and viral neuraminidase could be readily obtained with Ia-expressing L fibroblasts (5–8). This suggested that the fibroblasts possessed the capacity to process these antigens. This was also supported by the known antigen-processing requirements of some of these T cells (9). Nevertheless, T-cell clones specific for ovalbumin and lysozyme could not be stimulated by the same transfectant L cells (6, 7). The reasons for these failures could be attributed to differences between the T-cell interactions with the physiologic APC versus the L-cell transfectants, presumably due to their distinct differentiation lineages (leukocyte versus fibroblast). This difference could relate to any combination of parameters such as inadequate processing, lack of target structures for T-cell accessory molecules (L3T4, LFA-1, etc.), inability to provide sufficient lymphokine activities, etc., all known to be involved at different stages of the activation process (reviewed in ref. 19).

As the nominal antigen specificity of the T cells used by us was known to reside within amino acids 74–96 of HEL, we evaluated the ability of Ia-expressing L fibroblasts to process

Table 6. The E_β subunit determines the MHC specificity of I-E^k-restricted AOIC T cells

APC	I-E	[^{3}H]Thymidine incorporation, cpm $\times 10^{-3}$ per culture	
		Medium	T11(H)
CA36.1.3	$E^k_\alpha E^k_\beta$	8.0 ± 0.5	*145.0 ± 2.6*
CA36.2.1	$E^k_\alpha E^d_\beta$	6.6 ± 0.2	5.3 ± 1.0

Culture conditions were the same as in legend to Table 3.

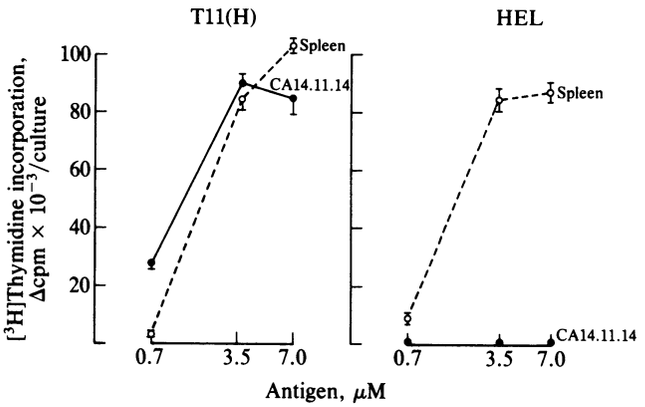

FIG. 1. T11(H)- versus native HEL-induced proliferative response of long-term KOIT.2.10 T cells. T cells were cultured in medium alone or in the presence of various concentrations of soluble peptide T11(H) or native HEL. Mitomycin C-treated CBA spleen cells (○) or I-A^k-transfected L cells, CA14.11.14 (●) were used as APC. Culture conditions were the same as in the legend to Table 3. Background incorporation with medium alone was 0.5×10^3 cpm for CBA spleen cells and 8.4×10^3 cpm for CA14.11.14 and has been subtracted. Bars indicate ±SEM.

and present this determinant present in the native HEL primary sequence to T cells. Fig. 1 compares the APC function of freshly prepared CBA spleen cells to that of I-A^k-expressing CA14.11.14 cells. While the T11(H) peptide was efficiently presented by both sources of APC, the transfectant L cells, in contrast to splenic APC, were incapable of presenting native HEL to the same T cells.

In this experiment, we had used spleen cells from the CBA strain as the source of APC because they are syngeneic with the CBA-derived T-cell clone KOIT.2.10. The L-cell fibroblasts are, however, derived from the C3H mouse. To address possible variations in the ability of APC from different mouse strains to present antigens to T cells (28), we compared APC from the C3H spleen and CA14.11.14 cells. To assess the possibility that this phenomenon was related to the fact that we had used long-term T cells, we employed a T-cell hybrid clone AOIT.H.2, similar to T-cell hybrids used in some of the other studies. This clone is specific for the same T11(H) peptide and is restricted by the I-A^k molecule (Table 1). Fig. 2 shows the IL-2 response of the T-cell hybrid cells cultured with either C3H spleen cells or CA14.11.14 cells in the presence of the T11(H) peptide, native HEL, or both. Again, the L-cell transfectants, in contrast to the syngeneic C3H splenic APC, completely failed to present HEL, even at a supraoptimal dose of 70 μM. Both cell types were capable of presenting the T11(H) peptide at similar concentrations. Furthermore, the inability to present HEL was not due to a selective inhibition of L-cell APC function, as shown by the negligible effect of inclusion of HEL in the T11(H)-stimulated cultures. It thus appears that L cells are unable to present the same T11(H) peptide when it is part of the native structure of HEL.

This result is analogous to the results obtained with several protein antigens, including lysozyme, using physiologic APC pretreated with lysosomotropic drugs or aldehydes (9–11). It should be emphasized that no such pretreatments were necessary with the L-cell transfectants. The possibility that mitomycin C pretreatment of the APC, carried out to prevent background proliferation, selectively interfered with the presentation of HEL was ruled out by using untreated transfectant L cells and AOIT.H.2 hybrid cells (data not shown). The similarity in the response phenotypes of pretreated physiologic APC and untreated L-cell transfectants suggests that the latter may be inherently compromised in their ability to process native HEL. It is interesting that these

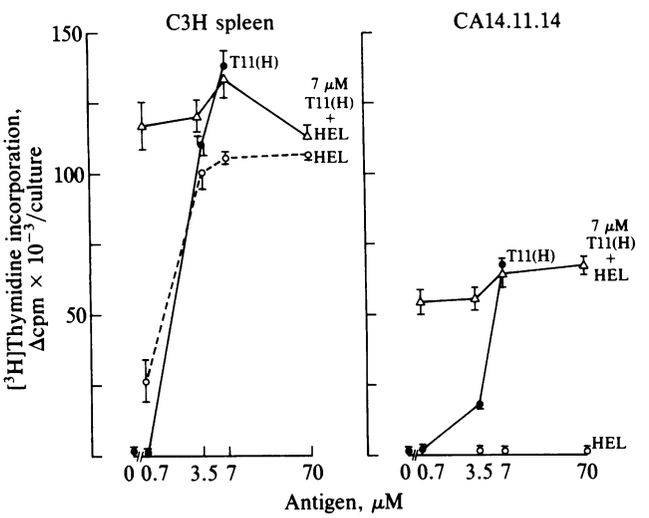

FIG. 2. Presentation of the T11(H) peptide and/or native HEL by C3H strain spleen cells or I-A^k transfectant CA14.11.14 cells to T-cell hybridoma AOIT.H.2. Culture conditions were the same as in the legend to Table 3 except that 1×10^5 T cells were used. Antigens were included at the indicated concentrations. T11(H) (●), HEL (○), and 7 μM T11(H) were included with indicated concentrations of HEL (△). Thymidine counts shown are those incorporated by the IL-2-dependent HT-2 cell line cultured with the 24-hr supernatant from each well of the primary culture and are a measure of the IL-2 secreted by the stimulated T-hybrid cells. Bars indicate ±SEM.

and similar transfectants have been shown to be capable of presenting other complex polypeptide antigens, such as keyhole limpet hemocyanin, poly(Glu, Ala, Tyr), and viral neuraminidase, to T cells under similar experimental conditions (5–8). It then follows from the known involvement of lysosomal degradation pathways in antigen processing that protein antigens may differ in the stringency of processing requirements. The fact that proteins differ widely in their susceptibility to proteolysis supports this contention (29).

In conclusion, Ia-expressing L-cell transfectants are likely to be very useful reagents not only in the analysis of structure–function relationships of the Ia molecule but also in the study of biochemical events associated with the phenomenon of antigen-specific MHC-restricted T-cell activation.

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